Institute of Health and Community Medicine

Cloning and Expression of Domain III, prM and NS1 Proteins of Zika Virus for Antigenicity Study

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

Zika virus (ZIKV) is a mosquito-borne virus that causes Zika fever and neurological complications such as Guillain-Barré syndrome and congenital microcephaly. ZIKV (family *Flaviviridae*, genus *Flavivirus*) is transmitted to humans through the bite of infected mosquitoes. Similar to other flaviviruses, ZIKV is a positive-sense, single-stranded RNA virus and has a 10,794 nucleotide genome. The genome consists of structural and non-structural proteins. The envelope (E) glycoprotein is the major structural protein of flavivirus which plays an important role in virus entry mechanism and is a major target for neutralizing antibodies. The E protein consists of three structurally distinct domains; domain I, domain II and domain III. Domain III (EDIII) of the E protein has shown strong neutralizing properties that can elicit antibodies. Precursor membrane (prM) glycoprotein is one of the structural proteins and it has been shown to form an unusual complex with the EDIII protein. This complex is responsible for virus assembly, fusion and also immunity-inducing of the virus. One of the non-structural proteins of flaviviruses, non-structural 1 (NS1) protein is currently a target for viral biomarkers as it is able to induce antibodies production during Zika infection. In this study, the EDIII, prM and NS1 regions of ZIKV were cloned and expressed using the pET SUMO expression system. Methods such as SDS-PAGE and Western blot were used to analyse the expression of each recombinant proteins. The recombinant fusion proteins were successfully expressed at their approximated molecular weights; EDIII (38.2 kDa), prM (20 kDa), NS1 (dimer 40 kDa and monomer 20 kDa). These proteins were then purified using nickel-affinity column chromatography and the reactivity of purified recombinant proteins were evaluated in immunoblot assay and indirect IgG ELISA. Twenty human serum samples were used in

both of the assays and the results of this study showed that the recombinant EDIII, prM and NS1 fusion proteins have different consistencies in the assays.

Keywords: Zika virus, gene cloning, recombinant protein, protein immunoassay

Pengklonan dan Pengekspresan Protein Domain III, prM dan NS1 Zika Virus untuk Kajian Antigenicity

ABSTRAK

Virus Zika (ZIKV) ialah virus bawaan nyamuk yang menyebabkan demam Zika dan komplikasi neurologi seperti sindrom Guillain-Barré dan kongenital microcephaly. ZIKV (keluarga virus Flaviviridae, genus Flavivirus) menjangkiti manusia melalui gigitan nyamuk yang dijangkiti virus tersebut. Serupa dengan flavivirus yang lain, ZIKV mempunyai RNA positif yang bebenang tunggal dan mempunyai 10,794 genom nukleotid. Genom tersebut mempunyai protein-protein berstruktur dan tidak berstruktur. Protein envelope (E) adalah protein berstruktur yang terbesar dalam flavivirus dan memainkan peranan penting dalam mekanisma kemasukan virus dan merupakan sasaran untuk antibodi yang mampu meneutralkan virus. Protein E terdiri daripada tiga domain iaitu; domain I, domain II dan domain III. Domain III (EDIII) telah menunjukkan ciri-ciri meneutralkan dan mampu mencungkil antibodi. Protein precursor membrane (prM) adalah salah satu protein berstruktur yang membentuk suatu kompleks dengan protein EDIII. Kompleks tersebut bertanggungjawab dalam perhimpunan virus-virus, penyatuan dan juga pendorongan imuniti oleh virus. Salah satu daripada protein tidak berstruktur dalam flavivirus, non-structural 1 (NS1) protein kini menjadi sasaran untuk viral biomarker oleh kerana kemampuan untuk mendorong penghasilan antibodi ketika jangkitan Zika. Di dalam kajian ini, region EDIII, prM dan NS1 dalam ZIKV telah diklon dan diekspres dalam system pengekspresan pET SUMO. Kaedah-kaedah seperti SDS-PAGE dan Western blot telah digunakan dalam menilai ekspresi setiap protein rekombinan. Protein-protein penyatuan rekombinan tersebut telah berjaya diekspreskan pada berat molekular yang dianggarkan; EDIII (38.2 kDa), prM (20 kDa), NS1 (dimer 40

kDa dan monomer 20 kDa). Setelah itu, protein-protein ini diaslikan menggunakan kromatografi nikel dan kereaktifan diuji dalam asai immunoblot dan ELISA IgG. Terdapat 20 sampel serum manusia telah diuji dalam kedua-dua asei tersebut dan keputusan menunjukkan bahawa protein-protein penyatuan rekombinan EDIII, prM dan NS1 mempunyai ketekalan yang berbeza dalam asei berkenaan.

Kata kunci: *Virus Zika, pengklonan gene, protein rekombinan, ujian immuno protein*

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LIST OF ABBREVIATIONS

C	capsid
CBB	Coomassie Brilliant Blue
cDNA	complementary DNA
DENV	Dengue virus
DNA	deoxyribonucleic acid
E	envelope
E. coli	*Escherichia coli*
EDIII	domain III
ELISA	enzyme-linked immunosorbent assay
GBS	Guillain-Barré syndrome
HPR	high dengue positive reference
IgG	immunoglobulin G
IgM	immunoglobulin M
IPTG	Isopropyl-β-D-1-thiogalactopyranoside
JEV	Japanese encephalitis virus
NS1	non-structural 1
OD	optical density
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PNR	pooled negative reference
prM	precursor membrane
PRNTs	plaque-reduction neutralization tests

rEDIII	recombinant EDIII
RNA	ribonucleic acid
rNS1	recombinant NS1
RO	reverse osmosis
rprM	recombinant prM
RT-PCR	reverse-transcription polymerase chain reaction
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SM	skimmed milk
SUMO	small ubiquitin-like modifier
UHQ	ultra-high quality
WNV	West Nile virus
ZIKV	Zika virus

CHAPTER 1

INTRODUCTION

1.1 Study Background

Zika virus (ZIKV) is a member of the virus family *Flaviviridae* and genus *Flavivirus.* Similar to other flaviviruses such as dengue virus (DENV), yellow fever virus and Japanese encephalitis virus (JEV), ZIKV is primarily transmitted by mosquitoes to humans. In general, these viruses causes millions of infections in the global population annually (Weissenbock et al., 2010). ZIKV was first isolated from a sentinel rhesus monkey in the forest of Zika, Uganda (Dick et al., 1952) and first human infection was reported from Nigeria in 1954 (Roth et al., 2014). Although ZIKV has been circulating in Africa and Asian countries for sixty years (Dick et al., 1952), but the massive outbreaks of Zika disease in Yap Island, Micronesia in 2007 caused a major shift in ZIKV epidemiology (CDC, 2016). Besides that, major outbreak of the disease surfaced in French Polynesia at the end of 2013 and a rise in neurological complications and Guillain-Barré syndrome (GBS) was reported (Oehler et al., 2014). The direct association of these complications to ZIKV could not be established as there was also the co-circulation of DENV (Roth et al., 2014).

Asian countries are known to be endemic to arboviral diseases and ZIKV infection were reported sporadically in the Philippines (Alera et al., 2015), Cambodia (Heang et al., 2012), Thailand (Buathong et al., 2015) and Indonesia (Olson et al., 1990). Furthermore, Malaysia is a dengue-endemic country and has favourable ecological conditions for ZIKV transmission. Although serological evidences of ZIKV infections were obtained from human samples in the 50s and 90s (Smithburn, 1954), till date there is no recent outbreak of Zika disease in Malaysia (Woon et al., 2019). An acute ZIKV infection was reported in

2014 in a German traveler returning from Sabah, Borneo. It was concluded that in Borneo, either the virus only rarely infects humans or it was possibly mistaken for dengue fever (Tappe et al., 2015). Similar clinical presentations between ZIKV illness and other arboviruses such as dengue and chikungunya may cause diagnostic confusion especially in regions where these viruses co-circulate (Duffy et al., 2009; Heang et al., 2012; Ioos et al., 2014).

1.2 Problem Statement

Generally, diagnosis of ZIKV infection is established either in direct method such as the detection of viral ribonucleic acid (RNA) by reverse-transcription polymerase chain reaction (RT-PCR) or by indirect method to identify the presence of ZIKV-induced antibodies (Waggoner & Pinsky, 2016). However, virus detection by RT-PCR is only feasible at the beginning phase of infection (Campos et al., 2015). Serological assays of ZIKV infection is challenging because of the cross-reactivity with other flaviviruses especially in endemic areas (Rabe et al., 2016). The similar antigenic determinants among flaviviruses resulted in species-specific and flavivirus cross-reactive antibodies when being infected with one flavivirus (Rathore & St. John, 2020). Due to this, a more specific and rapid immunodiagnostic tests is still in a great demand (Munoz-Jordan, 2017).

1.3 Objectives

The interest of this study therefore, is to investigate the application of the domain III of the envelope (EDIII), precursor membrane (prM) and non-structural 1 (NS1) proteins of ZIKV in the development of a serological assay for specific detection of ZIKV-induced

immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies. This can be achieved through the following set of objectives:

i. To clone EDIII, prM and NS1 region of ZIKV
ii. To express and purify recombinant proteins of EDIII, prM and NS1 of ZIKV
iii. To evaluate the potential of these recombinant proteins for antibody production

1.4 Chapter Summary

The focus of this study was to clone and express EDIII, prM and NS1 target regions of ZIKV. The regions were amplified using specific primers designed in this study and was cloned in *E. coli* cloning vector. Positive clones were screened by colony PCR and the selected clone was verified by deoxyribonucleic acid (DNA) sequencing. Then, the recombinant proteins were expressed and purified using nickel-affinity column chromatography. At the end of this study, the reactivity of the purified recombinant proteins were evaluated in immunoblot assays and also tested against human sera in indirect IgG ELISA.

CHAPTER 2

LITERATURE REVIEW

2.1 Zika virus

ZIKV is a member of the *Flaviviridae* family, in the genus *Flavivirus*. It was first isolated from rhesus monkeys in the forest of Zika, Uganda in the 1940s (Dick et al., 1952) and the first case of human infection was reported in Nigeria a decade later (Macnamara, 1954). Dengue virus (DENV) and Japanese encephalitis virus (JEV) are among the mosquito-borne viruses from the same family as ZIKV (Weissenbock et al., 2010). Every year, millions of infections caused by these viruses were reported across the globe (Weissenbock et al., 2010). There is no cure for Zika virus disease at the moment, and treatment is focused on relieving the symptoms. To date, the development of a ZIKV vaccine is still in progress (WHO, 2018).

2.2 ZIKV epidemiology

The first case of ZIKV infection in humans was recorded in Nigeria in 1954 (Macnamara, 1954). For decades, ZIKV is known to cause only sporadic human infections in Africa and Asia. The first major outbreak was reported in Yap Island, Micronesia in 2007 (Duffy et al., 2009). In October 2013, the second major outbreak of ZIKV occurred in French Polynesia, causing over 30,000 patients to seek medical attention in health-care facilities (Roth et al., 2014). During this outbreak, increased incidence of neurological complications and Guillain-Barré syndrome (GBS) was observed and this was being associated with ZIKV infection. However, this association requires further investigations as co-circulation of dengue virus was also reported at the time (Oehler et al., 2014).

Following the outbreak in French Polynesia, ZIKV infections were also reported in Cook Islands, New Caledonia and Easter Island, confirming the ability of this virus to spread outside of African and Asian regions (Roth et al., 2014). In May 2015, Brazil's National Reference Laboratory reported the first local infection of ZIKV and subsequent spread of the virus has affected about 1.5 million people (European Centre for Disease Prevention and Control, 2015a). With the exception of Europe, ZIKV has circulated to all other continents of the world (Boyer et al., 2018).

ZIKV infection has been described as sporadic in Asia, particularly Indonesia (Olson et al., 1990), Cambodia (Heang et al., 2012), Philippines (Alera et al., 2015) and Thailand (Buathong et al., 2015). In 2010, a 3-year-old Cambodian boy was reported with ZIKV infection of a strain closely related to the ones responsible in the Yap Island outbreak, both possibly share an ancestor that was isolated in Malaysia in 1966 (Boyer et al., 2018). This shows that the virus could be expanding its geographical distribution (Haddow et al., 2012). In Peninsular Malaysia, although ZIKV was previously detected in *Aedes aegypti* mosquitoes (Marchette et al., 1969) and antibodies against the virus were also detected in serum samples from patients (Smithburn, 1954), there is no recent report of Zika fever in the country. However, Tappe et al. (2015) reported an acute ZIKV infection in a German traveler after a trip to Sabah and Peninsular Malaysia in 2014. IgM and IgG to ZIKV were found to be present in the patient's blood through indirect immunofluorescence assay in the early stage of infection. Later on, ZIKV-specific neutralizing antibodies were also detected in viral neutralization testing.

The spread of ZIKV infection to the global population is similar to that of the other arboviruses such as DENV and CHIKV (Musso et al., 2015). It is common that ZIKV outbreak being initially misdiagnosed as dengue fever due to the similarity of symptoms